The frequency of 844ins68 mutation in the cystathionine β-synthase gene is not increased in patients with venous thrombosis

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Abstract

**Background and Objectives.** A frequent mutation in the cystathionine β-synthase (CBS) gene (844ins68, a 68-bp insertion in the coding region of exon 8) was recently discovered. In the present study we investigated this mutation as a candidate risk factor for venous thrombosis.

**Design and Methods.** The prevalence of the 844ins68 CBS mutation was determined in 101 patients with objectively diagnosed deep venous thrombosis and in 101 healthy controls matched for age, sex and race. PCR amplification of a DNA fragment containing exon 8 of the CBS gene was employed to determine the genotypes. Additionally, BsrI restriction enzyme digestion of the PCR products was performed in all samples from carriers of the insertion, to test for concurrent presence of a second mutation (T833C) in the CBS gene.

**Results.** The insertion was found in 21 out of 101 patients (20.8%; allele frequency 0.109) and in 20 out of 101 controls (19.8%; allele frequency 0.114), yielding a relative risk for venous thrombosis related to the 844ins68 CBS mutation close to 1.0. In addition, the T833C CBS mutation was detected in all alleles carrying the 844ins68 CBS insertion, confirming the co-inheritance of the two mutations.

**Interpretation and Conclusions.** Our findings do not support the hypothesis that the 844ins68 mutation in the CBS gene is a genetic risk factor for venous thrombosis.

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**Materials and Methods**

**Subjects**

Blood samples were obtained after informed consent from 101 consecutive patients (42 men and 59 women; mean age, 36 years; age range, 1 to 50 years) with a first episode of deep venous thrombosis objectively confirmed by phlebography or ultrasonography (color duplex scan). None of the individuals included in the patient group had evidence of malignant
DNA analysis
The PCR products were electrophoresed with the 844ins68 CBS insertion, differences in allele frequencies and an association containing exon 8 of the CBS gene as previously described. The PCR products were electrophoresed on a 2% agarose gel and photographed under UV light after ethidium bromide staining. The resulting fragments were 252-bp in the presence and 184-bp in the absence of the 844ins68 CBS insertion. Digestion of the PCR products with BsrI restriction enzyme was performed in all samples from carriers of the insertion, to test for concurrent presence of the T833C CBS mutation. Differences in allele frequencies and genotype distribution between patients and controls were assessed by the $\chi^2$ test and a $p$ value of 0.05 was taken as statistically significant.

Methods
Genomic DNA was extracted from peripheral leukocytes employing standard methods. DNA analysis was carried out by PCR amplification of a DNA fragment containing exon 8 of the CBS gene as previously described. The PCR products were electrophoresed on a 2% agarose gel and photographed under UV light after ethidium bromide staining. The resulting fragments were 252-bp in the presence and 184-bp in the absence of the 844ins68 CBS insertion. Digestion of the PCR products with BsrI restriction enzyme was performed in all samples from carriers of the insertion, to test for concurrent presence of the T833C CBS mutation. Differences in allele frequencies and genotype distribution between patients and controls were assessed by the $\chi^2$ test and a $p$ value of 0.05 was taken as statistically significant.

Results
Genotype distribution of the 844ins68 CBS mutation in patients with venous thrombosis and healthy controls is shown in Table 1. Twenty heterozygotes (19.8%) and 1 homozygote (1%) for the CBS insertion were found among 101 patients with verified venous thrombosis (allele frequency 0.109). In the control group, 17 heterozygotes (16.8%) and 3 homozygotes (3%) for the mutation were observed (allele frequency 0.114). These data yield an odds ratio for venous thrombosis associated with the CBS insertion close to 1.0, or a neutral relative risk for venous thrombosis linked to the mutant CBS allele. BsrI restriction enzyme digestion revealed that the T833C CBS mutation was present in all alleles carrying the 844ins68 CBS insertion.

Discussion
The description of new mutations in the CBS gene has stimulated the design of studies assessing the role of the specific genetic abnormality in vascular thrombosis and NTD. Recently, an insertion in the coding region of exon 8 of the CBS gene was identified as a frequent mutation in different populations in which its prevalence was investigated. Previous results for BsrI restriction enzyme analysis in carriers of the CBS insertion suggested that an additional mutation in the CBS gene (T833C) co-segregated in cis with the 844ins68 CBS insertion, an association also observed in all 68-bp mutated alleles identified in the present study. Taken together, these findings confirm the close association of both mutations and therefore the pattern of co-inheritance of the double T833C/844ins68 CBS mutation.

The 844ins68 CBS mutation apparently does not result in impaired enzyme activity or hyperhomocysteinemia, but mRNA data provided evidence that the allele carrying the insertion is poorly transcribed. The CBS insertion was investigated as a risk factor for arterial vascular disease in previous studies and controversial results were reported. The role of this genetic variation in arterial thrombosis, therefore, remains unclear. To our knowledge, the CBS insertion has never previously been investigated as a risk factor for venous thrombosis.

In the present study we determined the prevalence of the CBS insertion in patients with verified venous thrombosis and in healthy controls. We investigated a selected population of relatively young patients, in whom any influence of genetic risk factors for vascular thrombosis would be expected to be more easily detected than in older patients. We observed that the CBS insertion is highly prevalent in the Brazilian population, reaching frequencies similar to those previously reported for other populations. If the CBS insertion was indeed a risk factor for venous thrombosis, a higher prevalence of this mutation would be expected in the group of thrombosis patients in comparison with the control group. However, the mutation was found in a statistically identical prevalence among controls and patients, yielding a (neutral) relative risk for venous thrombosis linked to the insertion close to 1.0. This finding diminishes the likelihood that the CBS insertion is a risk factor for venous thrombotic disease.

The identification of genetic abnormalities which are of clinical significance and should be screened for in thrombotic patients is currently considered an important step for the management of venous thromboembolism. The findings from the present study do not support the hypothesis that the 844ins68 mutation in the CBS gene is a risk factor for venous thrombosis, indicating that screening for this genetic variation is probably not recommended for patients suffering from venous thrombotic disease.

<table>
<thead>
<tr>
<th>CBS genotype</th>
<th>Patients (n=101)</th>
<th>Controls (n=101)</th>
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<tbody>
<tr>
<td>N/N</td>
<td>80 (79.2%)</td>
<td>81 (80.2%)</td>
</tr>
<tr>
<td>N/I</td>
<td>20 (19.8%)</td>
<td>17 (16.8%)</td>
</tr>
<tr>
<td>I/I</td>
<td>1 (1%)</td>
<td>3 (3%)</td>
</tr>
</tbody>
</table>

None of the differences was statistically significant ($p$ values > 0.05). N/N: normal genotype, N/I: heterozygous for the CBS insertion, I/I: homozygous for the CBS insertion.
Contributions and Acknowledgments
RF was the principal investigator involved in the design of the study, analysis of the data, and interpretation. He wrote the paper with MZ, who was responsible for the general supervision of the investigation and its funding. All of the other authors played a part in the design and execution of the study. We are most grateful to Amélia G. Araújo, Marli H. Tavel-la and Walter J. Cassinelli for excellent technical assistance.

Funding
This research was partly supported by CNPq, FUNDHERP and FAPESP.

Disclosures
Conflict of interest: none
Redundant publications: no substantial overlapping with previous papers.

Manuscript processing
Manuscript received May 14, 1998; accepted August 21, 1998.

References